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# THE USE OF STAINS, ESPECIALLY WITH REFERENCE TO THEIR VALUE FOR DIFFERENTIAL DIAGNOSIS.

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To-day the powers of staining re-agents are well known, but the number of dyes is so great and the names so confused by different makers that unless the microscopist has all possible information as to the commercial and chemical name, trade-mark, reference number, or maker and firm from whom bought, the identification of the dye becomes almost impossible.

For example, you write for magenta or fuchsin, and receive *a* fuchsin, but your result is poor, because it is *acid* instead of *basic* fuchsin, the basic anilines being best adapted for micro-organisms.

The chemical nature of the dye is of great importance. Rosanilin hydrochloride and rosanilin acetate are both called in trade magenta or fuchsin, but the first (hydrochloride) will stain tubercle and leprosy bacilli and the acetate will not.

Letters are sometimes replaced by numbers, as B is now, I believe, number 39. The stronger the staining power the higher the letter or the number of letters, as BBB is stronger than B or BB, etc. These facts should be carefully noted when buying the stain and inserted in any description of its use in a new process of staining.*

*The object of staining* is to demonstrate the minute structure of any tissue. The sections require staining with some coloring agent that will differentiate the elements by the manner in which they absorb the coloring matter. An ideal stain is one which in aqueous solution will at one operation so stain the elements of a section that they will show distinctly under the microscope by their different reaction to it. Whatever may be the nature of the re-agents used, they have all this peculiarity, that by their aid special structures are brought out more prominently or one tissue is differentiated in appearance from the other tissue in which it lies.

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*One of the most useful small books for giving synonyms and the commercial relations of carbon colors is the "Tabellarische Uebersicht der Künstlichen organischen Farbstoffe," Schultz und Julius. Berlin, R. Gaertner.—W. H. S.

Thus it has been found that certain vital parts of cells are more deeply stained by most staining re-agents (carmine) than are the surrounding parts, or, again, a few re-agents (picric acid) have especial affinity for the formed material of the cell, or the cement substance may be especially picked out, as by nitrate of silver. Again, it is also necessary to stain to show the distribution of bacteria in the tissue, the manner in which it is affected, the extent of disease, and what parts are involved.

Stains for convenience may be classified into (1) *vegetable,* as logwood; (2) *animal,* as cochineal; (3) *anilines,* and (4) *metallic,* as nitrate of silver, etc.

In the first group logwood or hæmatoxylin is the best, in my opinion, as a good, safe, reliable, and pleasant stain, one which I always use as a counter-stain or check for comparison. Of the numerous formulæ, perhaps Kleinenberg's, Gibbes', Mitchell's, and Weigert's are the best. A stain made from the chips is to be preferred, as it is more uniform than from the extract and less adulterated. The best chips to use are those of a tawny color and not too small. The chief objection in making logwood stains is the *variation* of the stain, and this seems to me to be usually caused when the alum is added to the red fluid, the exact point at which to cease the addition of the alum being subject to variation and only gained through experience. Logwood renders the nuclei of the cells markedly conspicuous. The stratified epithelial cells are of a dark bluish tint, the fibrous tissue of a light gray or slate color, and it is a very good contrast stain. It is well to remember that specimens stained with logwood gradually lose their color in glycerine, and are therefore best preserved in Canada balsam. Weigert's logwood method is very good for the cerebro-spinal center. The white substance (medulla) of Schwann is sharply defined by a dark tinge; axis cylinders, ganglia, cells, nuclei, etc., remain almost colorless. The long postulated alteration of the gray matter of the cord in tubes or locomotor ataxia, which until this method was discovered had been sought for in vain, is now a sure demonstration. The alteration consists in the atrophy of the fine, reticulated nerve fibers within the column of Clarke. The result of this is of great theoretical importance; and similar changes, perhaps, remain to be discovered in the cortex cerebri in progressive paralysis in secondary degenerations, in retinal affections, etc. It forms a very good ground stain for double and multiple staining. Calcareous degeneration is stained reddish with logwood and sharply defined from the rest of the specimen by its color. In studying this process, pass a few sections through dilute acetic acid to remove the lime-salt deposit. This will show if the structures are normal or abnormal. In leucocythemic liver the leucocytes are of a much darker purple color, and they are easily picked out from the normal tissue, being especially numerous at the periphery of the lobules, by which they are very distinctly outlined and crowding the vessels, the blood in them being yellow, and the exudation of leucocytes shows very plainly among them and into the spaces between the liver cells, and in many places these appear pale and atrophied, even destroyed, by the pressure of these deeply stained leucocytes, though otherwise unchanged. In sections containing mucous glands logwood shows very clearly that there is a chemical change during secretion, and the state of inactivity by the difference in the staining of the cells, etc., in the disease "dermitokeras" (Hamilton), or what is apparently a very rare form of epithelioma, the pathological appearances of which suggest the presence of a disease similar to, if not identical with, that termed by Mr. Jonathan Hutchinson, of London, England, "arsenic keratosis" and "arsenic cancer," which he considers caused by the prolonged use of arsenic. (See Trans. of Path. Soc. London, vol. 39, p. 352 *et seq.*) The clinical history proves arsenic was *not* the cause in the specimen I have, although there is great similarity in the macroscopic appearances and the history of the two affections. Here logwood demonstrates two kinds of changes in the disease—irregular masses, which have a yellow color, and columns of epithelial cells, which permeate the sections, giving the appearance of a net-work of trabecules. The interstices between these is connective tissue of rapid growth. These yellow cells seem identical with the formation found in horny growths of the epidermis, and are concentric in arrangement and composed of cells undergoing horny change, but they differ from epithelioma cells undergoing horny change in that there is a sharp line of demarkation between these and the cells forming the columns of epithelial growth. The horny change is found throughout the whole mass and not as a gradual change. The important point in this case is the relation between the horny growths and the rapidly growing epithelioma. On examining a large number of sections the newly formed epithelial growth starts from the periphery of the horny mass. Some of these horny masses show a distinct rounded outgrowth at one spot in their periphery, and the value of the logwood stain is shown by its marking very plainly the starting point of this outgrowth. This specimen is very rare and I shall be pleased to show it to the members of the Society who may care to see it. Again, the value of staining is seen in "actinomycosis" taken from some cow's tongues and hardened in chromic acid mixture, Müller, and alcohol, but the last is not a good method. I will here mention that I have seen appearances presented in the liver and sputum so like this disease that they were regarded by several friends as identical, though I differed in opinion, on account of the action of staining and not from any ordinary examination, for I was undecided, I must own, until after the action of the dye proved the case. The rays are usually more frequent in the tissue under the epithelium, and much smaller than usually given in text-books, where they had caused local inflammatory action. The staining is as yet very unsatisfactory and the results poor, seldom, I believe, ever showing the appearance as given in Crookshank's "Bacteriology," which I regard as unusual, rather diagrammatic, in fact. In cattle these masses do not break down nearly so readily as in man. In cattle the positions in which the disease most frequently occurs are the jaws and first part of alimentary canal; in the human subject, the soft parts of the neck, the mediastinal tissue, and the lungs. By staining with Spiller and mounting in Canada balsam, the tissue stains, but not the fungus. With logwood the corpuscles, so-called Miescher or Rainey's, show a granular appearance, but with a Zeiss one-twelfth oil immersion some rod-like structures appear faintly. No other staining agents would bring out anything more definite. With double staining some of the substance in the corpuscles gave a different chemical reaction from the rest. Double and treble staining is of the utmost value in solving many difficult points in minute histology and pathology. Use colors that will not combine in the sections, but will differentiate various elements. Picro-carmine is about the best ground stain, and always stain deeply with it before trying other colors. In this way connective tissue, muscle, fibers, etc., are colored red, to begin with. But the contents of the corpuscles in the tongue were still unstained. Now came the task of getting a stain which would color the rod-like bodies so dimly visible with a high power in the corpuscles. After a repeated trial of a number of stains, Spiller's purple 2 per cent. in

$H_2O$ gave the rod a decided tint and did not affect the surrounding granular matter. In this manner the rods showed as quite separate from the granular matter around them. The inflammatory nodules in the subepithelial tissue and the rays were highly colored and exactly the same as the rod-like bodies in the corpuscles inside the muscle fibers, and appeared to be identical. The mycelium in the sections here stained a faint yellow tinge from the picro-carmine, and on being very closely examined the same color was seen in the granular matter of the corpuscles.

Of the *animal stains*, cochineal (Czoker) is a most useful stain and is, for many reasons, superior to borax-carmine. It does not matter what the material has been hardened in ; it requires no preparation, but can be put at once in the stain, and if left in too long the sections do not overstain. It is a useful ground stain for multiple staining, and, as it is a very transparent delicate color, some very beautiful combinations can be made with aniline colors. It acts quickly, and is therefore useful for class purposes, and, besides, is elective and permanent. Nuclei and axis cylinders stain a bluish-red color, protoplasm and connective tissue only faintly. Horny substances often have a yellowish tinge. To make the stain, rub up in a mortar 7 parts of cochineal (coccus cacti) with 7 parts of potash-alum, powdered. Add 700 parts of distilled water, and evaporate slowly to 400 parts. When cool add enough carbolic acid to be perceptible by smell, and filter several times. The solution is now ready for use and will keep for months, though it may require to be filtered again after some six or twelve months, and more carbolic acid added. It is used in the same manner as picro-carmine and mounted in Canada balsam.

Of the metallic dyes, silver nitrate and chloride of gold are examples and are especially useful for serous membranes, nerve endings, developing blood-vessels, and pigment corpuscles.

The *anilines* are very numerous and are especially employed because of their affinity for nuclear structures. As a general thing, the nuclei of the lymph cells, as well as those of the connective tissue and endothelial cells, are much more deeply stained than the epithelial nuclei. It is through this distinct isolated nuclear staining Weigert discovered in a large number of pathological processes that the cell nuclei disappear, either universally or at least in certain categories of cells. The absence of nuclei was first detected in the cells of the deep layers of the epidermis in small-pox ; then in diphtheria ; again in various organs in the neighborhood of colonies of micrococci, in the convoluted uriniferous tubules, in cases of chrome poisoning, in renal infarcts, cheesy degenerations, etc. The absence of nuclei occurs in necrosed cells which remain exposed for a certain time after death to the action of the nutritive juices which circulate, though in a lessened degree, through the lacunæ spaces. The protoplasm of the cells in many cases assumes at the same time a brightly glistening, homogeneous appearance, and called by Cohnheim "coagulation necrosis." This has often been misapplied, but should be limited to cases where coagulation with necrosis has been demonstrated. Before the nucleus completely disappears small deeply stained granules are often found in its place and are probably the remains of the disintegrated nucleus often erroneously called by beginners micrococci. Besides the nucleus, the dyes stain (1) the matrix of hyaline cartilage, amœboid cells in developing cartilage; (2) mucous substances, and particularly the mucous contents of glands; (3) most forms of micrococci and bacilli; (4) certain protoplasmic granulations—the protoplasm of the so-called nutritive cells.

For differential staining in *amyloid degeneration* we have recourse to safranine 1 per cent. in distilled water, vesuvin (5 per cent.), rubin 5 per cent. in distilled water, double staining with a number of combinations, all of which give most excellent results. To get the best results the material should be hardened with chromic acid, and in kidney it is very well brought out by this method. To stain the amyloid substance and differentiate it from the surrounding tissue the aniline dyes are mostly used, as they seem to have a special affinity for this degeneration. Safranine must be used till the sections are deeply stained, and then washed well in spirit until a peculiar red-brick color has left them, not long enough to wash the stain out in the spirit. The healthy tissues stain a fine rose color, the amyloid degeneration a fiery orange yellow. They can then be further stained in methyl blue with a very nice result, or stain first with logwood and then 1 per cent. solution of safranine in distilled water. Safranine and indigo-carmine give good results. Staining the sections first with a 1 per cent. aqueous solution of safranine, wash this out slightly and then stain with a 5 per cent. solution of indigo-carmine in water; wash again in water, then spirit with care, or the aniline will be removed; pass quickly but carefully through clove oil, and mount in Canada balsam. The amyloid degeneration is a deep blue, nuclei of the cells red, and the surrounding tissues a light blue. Carmine and indigo-carmine give a good result, the blue showing the amyloid degeneration in contrast to the rest of the tissue to perfection. Indigo-carmine with Bismarck brown is also a useful double stain, but not so good as rosanilin hydrochloride and *iodine* green or eosin and aniline blue. Rubin gives very elegant results and is a quick and useful stain. If left long enough in the stain it does not wash out so easily in the spirit. Methyl violet and gentian violet are both good, the amyloid and waxy substance being red—for it has the power of stopping violet rays, while the red pass through—the healthy tissues blue. Methyl green also colors hyaline casts ultramarine blue; so in a section of the kidney the healthy tissue would appear green, hyaline casts blue, and amyloid spots violet, no matter how small they may be.

By the employment of this method Eberth has shown that the cellular elements of the liver, kidneys, and spleen, as well as the smooth muscular fibers of vessels, are never subjects of amyloid degeneration, but that this always affects the intercellular substance and homogeneous membranes only. As yet I do not think the real nature of this disease is known, whether it be a simple deposit or a local degeneration, but on examining many sections, treated in all possible ways, it seems more a local degeneration. In the kidney the process is often seen in its earliest stage, and there the vessel at first is no larger in diameter than the normal; but in the liver, in advanced stages, the masses of amyloid material are usually larger than a normal liver cell, and on close examination it would seem when the disease once started it continues to increase from its point of origin, and, if so, must be a *local* degeneration, and usually occurs in the capillary vessels, and is well brought out by special stains. This degeneration is generally found associated with fatty infiltration, and where the liver is enlarged and much altered, sections will consist almost entirely of fatty and amyloid matter, and the former is easily shown by cyanin or quinolein blue, as it is an excellent test for fatty matters. This is a disease which if it had not been for the power of selective and double stains would as yet have been almost wholly undescribed, and illustrates the use and value of careful staining. The methyl-violet method must be used with caution, for other hyaline formations, also those possibly allied to the amyloid substance, as certain urinary casts, for example, exhibit the reaction, though they cannot be regarded as amyloid. Iodine and hydrosulphuric acid combined is a good and extremely delicate test for waxy material, but unfortunately does not succeed in every case. Iodine alone is a well-known test. Sections of amyloid degeneration may be stained with methyl violet and then washed in a saturated solution of oxalic acid, which discharges most of the color from the normal tissues, leaving them a dull slaty gray, but intensifies or brightens the red-violet stain of the waxy tissues. I find that methyl green shows the amyloid parts violet, the normal substance, and particularly the nuclei green. It shows the amyloid change in the walls of the small arteries in the spleen very well. Borax-carmine with indigo-carmine is an excellent stain, but requires experience in handling. By it all the amyloid is blue, nuclei of normal cells red, and the remaining tissue slate color.

In many sections there is to be noticed a marked difference in the reaction of staining agents to many of the cells, pointing out some chemical change. This is well shown in cystic ovaries and in specimens stained with chloride of gold and then hardened in spirit. What the action of gold chloride is on those parts it does not stain I do not know, but that it has some is evident from the difference of the action of aniline dyes on gold-prepared specimens and those hardened in any other way. If sections are made of *lymphatic glands in cases of scrofulous enlargement* a good example of the value of staining for differential diagnosis is found. A section from the gland shows two different kinds of cells by their reaction to staining agents, and the section is mapped out in areas where the cells stain faintly and between these irregular collections of cells that have stained deeply. It is plain that nearly all the original structure of the gland has been altered, and examination shows this has been done by the growth in it of these cells which stain faintly. The deeply stained cells are normal cells of the gland which remain unabsorbed, numerous in some parts compared to others, according to amount of new growth, but are always in the periphery; therefore this shows that the new growth commenced in the center or thereabouts of the normal follicular tissue. Giant cells are found, and if treated with special agents a few bacilli will give the specific reaction of Koch's tubercle bacilli; but they are smaller and shorter than those of caseous phthisis, and show a peculiarity which gives them some resemblance to the bacilli found in bovine tuberculosis, as well as their small size. If the gland material is kept in spirit for three or four years and sections made and stained to show these bacilli they will come out brightly, but in 24 to 48 hours they will have faded out entirely. Sections of lung with pulmonary phthisis, even if kept in spirit four or five times as long, when stained with the scrofulous gland in the same stain *retain their color* a long time. This fading of the bacilli occurs in laryngeal phthisis with Ziehl Neelsen's stain and in anthrax tissue kept in spirit; the bacilli will not stain in Spiller or gentian violet, and why as yet I do not think is known. The *combinations of double and treble staining* are many and varied and give different results, according to the organ, the hardening agent used, and the combining of the stain, one color displacing a part or all the color of another. Logwood and picro-carmine is a very old and useful stain, though care has to be used in the selection of the logwood, the color of some being so blue as to render the combination too diffuse or dark and not selective enough. To use, stain the sections in picro-carmine, then place them in plain filtered water acidulated with a few drops of picric or acetic acid for an hour or so; then change the sections from this to dilute logwood stain, using five to seven drops in a watch-glass of distilled water; do not let them stain too deeply; then wash *well* in filtered tap-water; change to spirit for ten minutes; clove oil ten minutes; mount in Canada balsam. The use of acidulating the water is to aid the sections to take the second stain better and not fade. It is very good for fresh tissues as well as for serous membranes; it brings out the connective-tissue corpuscles in the mesentery of the newt, and at the same time shows excellently the non-striped muscle. In tissues where there are various elements, as in developing bone, the scalp, and teeth, a vertical section through the nose showing development of the Schneiderian membrane, bone, and teeth, it is an excellent stain. If picro-carmine is used alone it may be a single or double stain, accordingly as used. When the section is stained, washed well in water, the picric acid is all removed; it acts as a single stain. If, on the other hand, it is quickly washed in alcohol the picric acid remains and there is a *double* combination. When making experiments in treble staining a number of sections may be stained in picro-carmine and placed in methylated spirit or alcohol and may remain until required, as the stain is not affected. Take some well-hardened sections of rodent ulcer and epithelioma and stain some in picro-carmine and logwood and others with rosanilin and iodine green and compare the two. In this way some definite result may be worked out, which, by using other specimens of the same disease, may be confirmed.

*Indigo-carmine and borax-carmine.*—The sections should be hardened in chromic-acid mixture (1/6 per cent. 2 parts, and 1 part spirit), as they do not require to be passed through a solution of carbonate of soda. Have the three following solutions ready for use, made as given:

(A) *Borax carmine*, ½ part; borax, 2 parts; distilled water, 32 parts; mix, decant, but do *not* filter. Now, stain in this for a few minutes, then wash in (B) hydrochloric acid, 1 part; absolute alcohol, 20 parts, until the tissues are a bright rose color, which only takes a few seconds; then wash in several changes of spirit to *remove* all acid. Now place in (C) a watchglassful of undiluted indigo-carmine, which is made as a saturated solution of the powder in distilled water, and filtered. A portion of this solution is then added to some alcohol or methylated spirit until it has attained a moderately deep-blue color; this must be filtered to remove the coloring matter which is precipitated; it is then ready for use. The filtered solution should not have a very deep-blue color, and when held to the light should show a purplish tint. It only takes a few seconds to stain the sections evenly. Wash in distilled water, pass through spirit, then cloves, into balsam.

N. B.—If the sections are left too long in the acid, the carmine is taken out at the edges and these parts will take on the blue stain too deeply. If the acid solution is too strong the tissue is affected as with a weaker solution. For carcinomata it is very good, as the carmine picks out all the new growths, and hardened in chromic acid it does away with passing the specimens through a solution of anhydrous bicarbonate of soda. This is a modification of Dr. Seiler's method in the American Quarterly Microscopical Journal.

To show the blood-vessels in the web of a frog's foot, the tail of a tadpole, and similar objects, carmine and indigo-carmine does away with injecting them and shows the vessels in a natural state. For combination staining with anilines, the principle worked on is to make a *spirituous* solution of one color and a *watery* solution of another; so make solutions of roseine and aniline violet in spirit and aniline blue and iodine green in distilled water. Use a few drops of one of the spirit solutions and stain for a short time, until deep enough, when they may be taken from the stain and examined in clean spirit; then washed, if deep enough, till no more color comes away in clean spirit, and then remove to a few drops of an aqueous solution, diluted, and place in the section from the spirit. When stained as desired, wash well in plain water and then place in spirit, when usually the first color comes away, and wash till quite clean, transfer to spirit cloves, and mount.

To stain with more than two colors, use gold chloride or picro-carmine with any of the anilines. A rat's tail is a very good tissue to stain with gold; then the anilines as described; also sections of the base of the tongue in different animals containing mucous glands. Perhaps the best combination is with picro-carmine and rosanilin, hydrochloride and iodine green. This gives a most elegant effect when used to isolate gland tissue, as Peyer's patches, glands of the tongue, œsophagus, Brunner's glands in intestine, the glands being green and the rest of the tissue red. If this result has not been obtained, then the sample was *methyl* and not iodine green.

Iodine green is an expensive color to prepare, and is in consequence not often found in commerce, methyl green superseding it. Fortunately to the microscopist this is no impediment, on account of the small quantity of the substance required for staining, and it will be found one of the most useful of the aniline stains. Methyl green is practically of little or *no* value to the histologist, and every care must be taken to ensure the dye being true to name, methyl being often supplied on account of the price and scarcity. Methyl green, known formerly as "vert en cristaux," is *not* a derivate of rosanilin, but obtained by treating methyl violet with methyl nitrate, while iodine green or Hoffmann's green is the hydriodide of tetra-methyl-rosanilin-methyl-iodide.* The stain is not destroyed by immersion in alcohol for days, while methyl green is dissolved. The samples of iodine and methyl green may be tested through the presence of iodine in one and not in the other. Take a little of the solid coloring matter and treat it with sulphuric acid, and then add a few small fragments of bichromate of potash; the iodine, if present, escapes in the form of violet vapors. It may also be demonstrated by means of chloroform or carbon sulphide ($CS_2$). The color may be had of excellent quality from C. A. F. Kuhlbaum's Chemische Fabrik, Berlin, S. O. (Zool. Anzeig., No. 130, 1883, p. 56). The great value of iodine green is that, besides being a nuclear stain, it has a great rapidity of action and a marvelous power of marking out by staining in various hues the different forms of tissue. For example, in a section of uterus of a roe deer and guinea pig the epithelial cells are blue, the glands dark green, and the muscle fibers malachite green, while the connective tissue re-

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*Chemistry of Iodine Green. Hoffmann & Girard, Ber. chem. Gesell, 440; Hoffmann, *ibid.*, vi, 552; Hoffmann, Monatsbericht der Königl. Acad. der Wiss zu Berlin, July 15, 1869.

mains unstained. For spinal cord it is very useful; also in kidney and skin.

Eosin and methyl blue are good, but the sections should be thin and only immersed for a few seconds in aniline blue. In sections from a case of dysidrosis the duct of the sweat glands was an entirely different color from the surrounding tissues, and so demonstrated its relation to the vesicles and showed some chemical change. I find the following combinations very good for staining *liver*, especially in inoculation cases: Magenta (rosanilin hydrochloride) and then iodine green, magenta, and logwood. For *heart*, to show the muscle and nuclei well, picro-carmine and iodine green. *Suprarenal capsule*—magenta and iodine green, picro-carmine and iodine green, Bismarck brown and picro-carmine, picro-carmine and methyl blue. Spleen—picro-carmine and logwood, magenta and logwood, magenta and blue. Kidney—Bismarck brown and iodine green, and Bismarck brown and picro-carmine.

The value of stains, and especially for differential diagnosis, is very well shown in demonstrating Kuhne and Lea's researches in the structure of the pancreas of the rabbit, in which they describe accumulations of cells which occur between the alveoli, and having a distinct blood supply. These, as proved by Prof. H. Gibbes, are constant in some animals, as the guinea pig, cat, dog, and ape, and lie among the alveoli in contact, but not mixed indiscriminately with them. Each accumulation consists of polyhedral cells, each having a distinct nucleus, which is irregular in shape and stains very deeply. The behavior of staining re-agents is a special feature in these cells. With logwood the only difference is that the nuclei color very deeply, much more so than the nuclei of the alveolar cells; but with other stains these cell masses can be stained a different color to the alveoli surrounding them, proving the difference in the cells. The stains are vesuvin and sulphindigotate of soda. Hardening the pancreas in chromic acid ⅙ per cent., 2 parts; spirit 1 part, gives the best results. Alcohol-hardened specimens are so delicate they will not bear the different processes. Place the sections in a watery solution of vesuvin for ten minutes—a 10 per cent. solution in distilled water and diluted one-half is the best—wash the sections well in distilled water till all the superfluous color is got rid of, then place in a 5 per cent. solution of sulphindigotate of soda until they become a deep-blue color; wash well in distilled water, then spirit, and mount in Canada balsam. It takes some practice to get the right shades of color, but when attained the difference between the two tissues is most marked, and shows that different portions of the pancreas are in different states of activity, thus mapping out the different zones very well.

In the grouse and golden pheasant, which are usually considered to be subjects of "avian tuberculosis," the liver was affected, and on staining sections with logwood, here and there patches of large cells were seen. These patches were composed of cells stained very differently both with logwood and methylene blue; those in the periphery took the stain well and showed one or more well-developed nuclei, while those toward and in the center stained faintly or not at all. Now, what was the origin of these cells? Well, after examining a large number of specimens from various cases, the conclusion arrived at was they were simply liver cells that had become hypertrophied. On staining sections with special stains I found those cells which had taken the logwood or blue stain well contained no bacilli, while those that had stained faintly or not at all with logwood or blue showed with the special stains several red bacilli resembling the tubercle bacillus—that is, the normal cells contained no tubercle bacilli, only those that were undergoing degeneration. Often you can find caseous masses in the liver of the domestic fowls and turkeys which contain large numbers of bacilli, staining similarly to the tubercle bacillus and resembling it somewhat in shape and size. Again, do we not get the characteristic red color of phthisis in lepra bacillus, bovine tuberculosis, scrofulous glands, and in the liver of a bird (the Rhea)? In this last the cells being completely filled with minute bacilli, owing to the smallness of the bacilli and the crowding and with a low power, the bacilli appear like dots. In the color, size, distribution, and character of the bacilli there exists a remarkable similarity between the nodules in leprosy and the nodules in glanders, as described by Schütz and Löffler in 1882,* and therefore can we now honestly claim all the value that was formerly given in diagnosis to the finding of bacilli stained red in phthisis? Are they the true sign of phthisis or only showing that caseation or necrosis has taken place and non-reticular tubercles formed; and, if so, then what are they in leprosy and the cases above quoted? To return to the bacilli in the liver of the birds, are they the bacilli of phthisis; and, if so, where do these seed-eating birds obtain the infection—from their food and surroundings, the earth in their runs, and the worms contained in the earth, or where? As a final example of the value of staining carefully, take the changes that take place in the lymphatic glands on the introduction of cancer cells for the formation of secondary growths in cancer.

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*Deutsche Med. Wochenschrift, 52, 1882; Klein, Micro. Organisms, 3rd edition.

Of course, we must take these glands when the process is in its commencement. Every care must be taken that the hardening is thoroughly done, little handling of the tissue, and the agents to be recommended are chromic acid, 1/6 per cent., 2 parts, and 1 of spirit; stir; change this fluid every second, fifth, and eighth days. On the ninth day use 2 parts of spirit and 1 water; stir; tenth day pure spirit or methylated spirit; if desired to cut on the fourteenth day, plain water; fifteenth, mucilage; sixteenth, cut, stain, and mount. If the tissue cannot be cut small, for the reason we wish to photograph or keep the material whole for class or museum purposes, Müller's fluid is the best, and it does not interfere with examining the sections for bacilli as does chromic acid. For *rapid* diagnosis, weak and then strong alcohol can be used, though as a hardening agent I never use it if I can avoid it. The value of the first two agents is that the specimens retain their natural bulk, the shape and size of their cell elements, and more nearly resemble the natural appearance of the tissue than with any other agent. In sarcomas and, indeed, where accuracy of diagnosis regarding the cells is required, spirit-hardening causes such contraction and shrinkage, even with every care, as to render the difference between large and small celled sarcomas and even some spindle-celled cancers a matter of some risk.

I have found a very good hardening agent made by using Müller's fluid and replacing the soda sulphate by cupric sulphate, using half the quantity of the soda, and in some things it is superior to the original Müller. I will here mention listerine as likely to be a useful agent in micro-work, though more especially for protozoa and the lower forms of pond life. A solution of listerine, 6 per cent., I find retards the ciliary motion of vorticellæ and all ciliated infusoria, and a strong solution is immediate death to all the lower animals. It promptly arrests the motion of such algæ as diatoms, oscillatoræ, etc., and also the motion of sponges during the process of "spore formation." It is also of use in gum for freezing tissue to keep it from bacteria and mold formation, etc., if used in from 30–33–50 per cent. solutions. Animal tissues are preserved in this re-agent; if used full strength, they undergo no putrefaction. A 10 per cent. solution prevents bacterial formation in the tissues. To return to our secondary cancer: After hardening and staining with logwood, we find three distinct forms of cells: (1) the normal lymph cell; (2) large cells with well-marked nuclei and showing very plainly the intracellular net-work; (3) large masses of homogeneous material, containing many irregularly shaped nuclei or, in other words, multinucleated cells. The lymph cells are unaltered. The large cells correspond exactly to the primary-growth cells and show a marked difference in logwood stain to the normal lymph cells. These stain deeply, the cancer cells only staining faintly. The multinucleated cells also stain deeply. By using multiple staining you can make out the relation of these cells to each other. Therefore choose picro-carmine for connective tissue, rosanilin sulphate with iodine green for the cells. The result is normal cells stained a bluish green by the combination of the last two colors. The cancer cells show a pure green, the multinucleated cells purple. In some of these cells small masses of protoplasm were separating, each portion of which always contained a nucleus and intranuclear net-work, and all stained green like the single cancer cells and not purple, like the mass from which they separated. With a one-twelfth Zeiss immersion these giant cells showed most of the nuclei irregular in outline, stained deep purple, and here and there you could see one of a more rounded outline and only stained faintly green. These were gradually becoming differentiated from the mass.

From this multiple staining one is justified in thinking a cancer cell reaching a lymphatic gland divides, forming a giant cell. Later, one of the nuclei separates and forms an independent cancer cell in the mobile or resting stage. Soon it becomes active, germinates, produces another giant cell; and, moreover, the chemical reaction is different in these two stages, as is seen by the affinity of one for the green and the other for the purple stain. During this time the fibrous stroma is increasing, gradually the normal structure is lost, and the gland becomes irregular, hard, and increased in size. In other organs where secondary growths occur, as in the liver and lungs, these processes can be seen. For the differential points in histology, take a section through the papillæ and base of the tongue and try multiple staining.

To succeed with these processes certain precautions must be attended to: Good and thorough hardening, with as little handling as possible, and the careful cutting and changing from water to spirit. The stains must be true to name, carefully prepared, and iodine and *not* methyl green used, as is so often the case; the careful labelling of the slides and entry of the *modus operandi* in a note book kept for the purpose.

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